

EXHIBIT

StressGen Biotechnologies Corp.
Molecular Biology Projects

Name of Construct: pTrc65/LF(4N+1C)
Start Date:
Lab Book#: AG-5, Page 27
Primary Researcher: Bill Wu & Cor Turnnir

Description:

4 repeats of P1A epitope (4 x LPYLGWLVF) was fused to the N-terminus of BCG Hsp65 gene in pTrc65/LF. The resulting plasmid expresses Hsp65 fused at its N-terminus with 4 repeats of P1A and at its C-terminus with 1 repeat of P1A

Cloning Procedure:

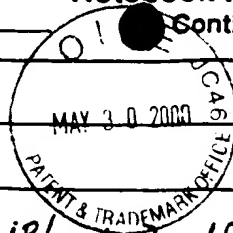
Two oligos coding for 5 repeats of P1A and a thrombin cleavage site were synthesized. Sense strand: cat ggc tct gcc gta tct ggg ctg gct ggt gtt cct gcc gta tct ggg ctg gct ggt gtt cct gcc gta tct ggg ctg gct ggt gtt cct ggt tcc gcg tgg atc. Antisense strand: cat gga tcc acg cgg aac cag gaa cac cag cca gcc cag ata cgg cag gaa cac cag cca gcc cag ata cgg cag gaa cac cag cca gcc cag ata cgg cag agc.

These two oligos which contain NcoI overhang on both ends were annealed and re-amplified with W066 (cta agt gcc atg gct ctg ccg tat ctg ggc) and W067 (agt cta agc cat gga tcc agc cgg aac cag). The PCR product was digested with NcoI, purified and ligated to pTrc65/LF DNA which had been previously digested with Nco I. The ligation mixture was transformed into *DH5α* and putative clones containing P1A N-terminal insert in the right orientation was selected by miniprep screening. The PCR product that was successfully amplified and inserted contained 4 repeats of P1A instead of 5 repeats. Maxiprep DNA was prepared and confirmed by diagnostic restriction digestion. Plasmid DNA was transformed into appropriate *E. coli* strain for expression analysis. The location of the fusion gene within the construct is illustrated on the following plasmid map.

Prepared by:

Date: _____

— Larry's sample.



① STD. BCG 65, 97. 98. 99. 100. 101. 102. 103. 105. 106 107.
108 p8150.

② BCG 65, STD. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120.
p8150

all 5ml loaded except p8150 (wml 7).

— Set up PIA.

	①	②	③	④	⑤	⑥
wob 4 (new)	2	2	2	2	2	/
wob 5 (old).	2	2	2	2	2	/

10X buffer	10	10	10	10	10	10
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10 mM DTP	2	2	2	2	2	2
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wob 6	1.0	/	1.0	1.0	/	1.0
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wob 7	/	1.0	1.0	1.0	/	1.0
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pfa pd.	0.5	0.5	0.5	0.5	0.5	0.5
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SDW	82.5	82.5	81.5	81.5	83.5	85.5
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Program #74. (96°C. 68°C. 72°C)

Continued on Page

Read and Understood By

Bill Wu.

Signed

Date

Lee M. M. M.

Signed

Date

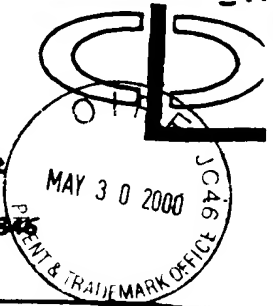
REQUEST FOR DNA SYNTHESIS

P.O. Number _____

Dalton Chemical Laboratories Inc.
119 Farquharson Bldg, 4700 Keele St
North York, Ontario, M3J 1P3
Tel: (416) 736-5394, Fax: (416) 736-5845
Attn: Peter Pekos

Order Date _____

Date Required _____



Customer Information

Dr. Bill W. Stresten
Name

#120-4243 Glenford Ave.
Address

Victoria B.C. V8Z 4B9
City Province Post/Zip

Tel. (604) 744-2811

Telephone Fax (604) 744-2877

Billing Information P.O. Attached _____

Stresten
Billing Name

#120-4243 Glenford Ave.
Billing Address

Victoria B.C. V8Z 4B9
Billing City Province Post/Zip

Special Instructions

Desalted DNA oligo without HPLC or PAGE purification

No. of OD units required 5-10

Sequence 1 Sequence Name WCB4 (159 mer)

5' (P) C A T G G C T C T G C C G T A T C T G G G C T G G C T

G G T G T T C C T G C C G T A T C T G G G C T G G C T

G G T G T T C C T G C C G T A T C T G G G C T G G C T

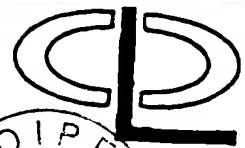
G G T G T T C C T G C C G T A T C T G G G C T G G C T

G G T G T T C C T G C C G T A T C T G G G C T G G C T

G G T G T T C C T G G T T C C G C G T G G A T C

5 repeats for mastoparan peptide (LF). w/ thrombin cleavage site. NoI at both ends

REQUEST FOR DNA SYNTHESIS

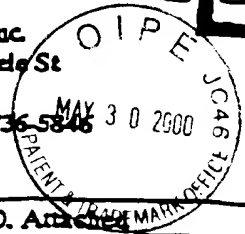


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Dalton Chemical Laboratories Inc.
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North York, Ontario, M3J 1P3
Tel: (416) 736-5394, Fax: (416) 736-5846
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Order Date _____

Date Required _____



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Sreesthen
Billing Name

#120-4243 Glenford Ave.
Billing Address

Victoria B.C. V8Z 4B9
Billing City Province Post/Zip

Special Instructions

Desalted DNA oligo without HPLC or PAGE purification

No. of OD units required 5-10

Sequence 1 Sequence Name W065 (159 mer)

5' (P) C.A.T G.G.A T.C.C A.C.G C.G.G A.A.C C.A.G G.A.A C.A.C

C.A.G C.C.A G.C.C C.A.G A.T.A C.G.G C.A.G G.A.A C.A.C

C.A.G C.C.A G.C.C C.A.G A.T.A C.G.G C.A.G G.A.A C.A.C

C.A.G C.C.A G.C.C C.A.G A.T.A C.G.G C.A.G G.A.A C.A.C

C.A.G C.C.A G.C.C C.A.G A.T.A C.G.G C.A.G G.A.A C.A.C

C.A.G C.C.A G.C.C C.A.G A.T.A C.G.G C.A.G A.G.C

5 repeats for mastomys peptide (LF). oligo ②.

STRESS GEN

BIOTECHNOLOGIES CORP.

#120 - 4243 Glenford Avenue, Victoria, BC, Canada V8Z 4B9 Tel: (604)744-2811 Fax: (604)744-2877



OLIGO REQUISITION FORM

Required by Bill Wu Month Day Year

Sequence (PLEASE PRINT CLEARLY in triplet form, direction 5' to 3')

5' AGTCTA AAC CAT GGA TCC ACG CGG AAC CAG 3'
1 30
Nco I
31 60
61 90
91 120
5' 121 150 3'

Sequence name W067 (name that means something to you)

Sequence length 30 mer

Comments: PCR primer (2) For LFS algo. Reverse.

HPLC purified, lyophilized oligo ug = pmols

SYNTHESIS#

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OLIGO REQUISITION FORM

Required by Bill Wy Month Day Year

Sequence (PLEASE PRINT CLEARLY in triplet form, direction 5' to 3')

5' CTA AGT GCC ATG GCT CTG CCG TAT CTG GGC 3'

1 30

31 60

61 90

91 120

121 150

5' 3'

Sequence name W066 (name that means something to you)

Sequence length 30 mer

Comments: PCR Primer ① For LFS algo. For World

HPLC purified, lyophilized oligo _____ ug = _____ pmols

SYNTHESIS#